MYELOFIBROSIS
THE ITALIAN EXPERIENCE

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Myelofibrosis in the eighties

- A poorly characterized biological syndrome
- Heterogeneity of clinical presentations
- No effective therapies

Erythrokinetic Classification of Myelofibrosis

(G. Barosi et al, BJH 1981, 26 cases from our Institution)

Class I (42%)

- Highly expanded erythropoiesis
- Centrifugal active marrow displacement
- Red cell mass normal or increased
- Ineffective erythropoiesis

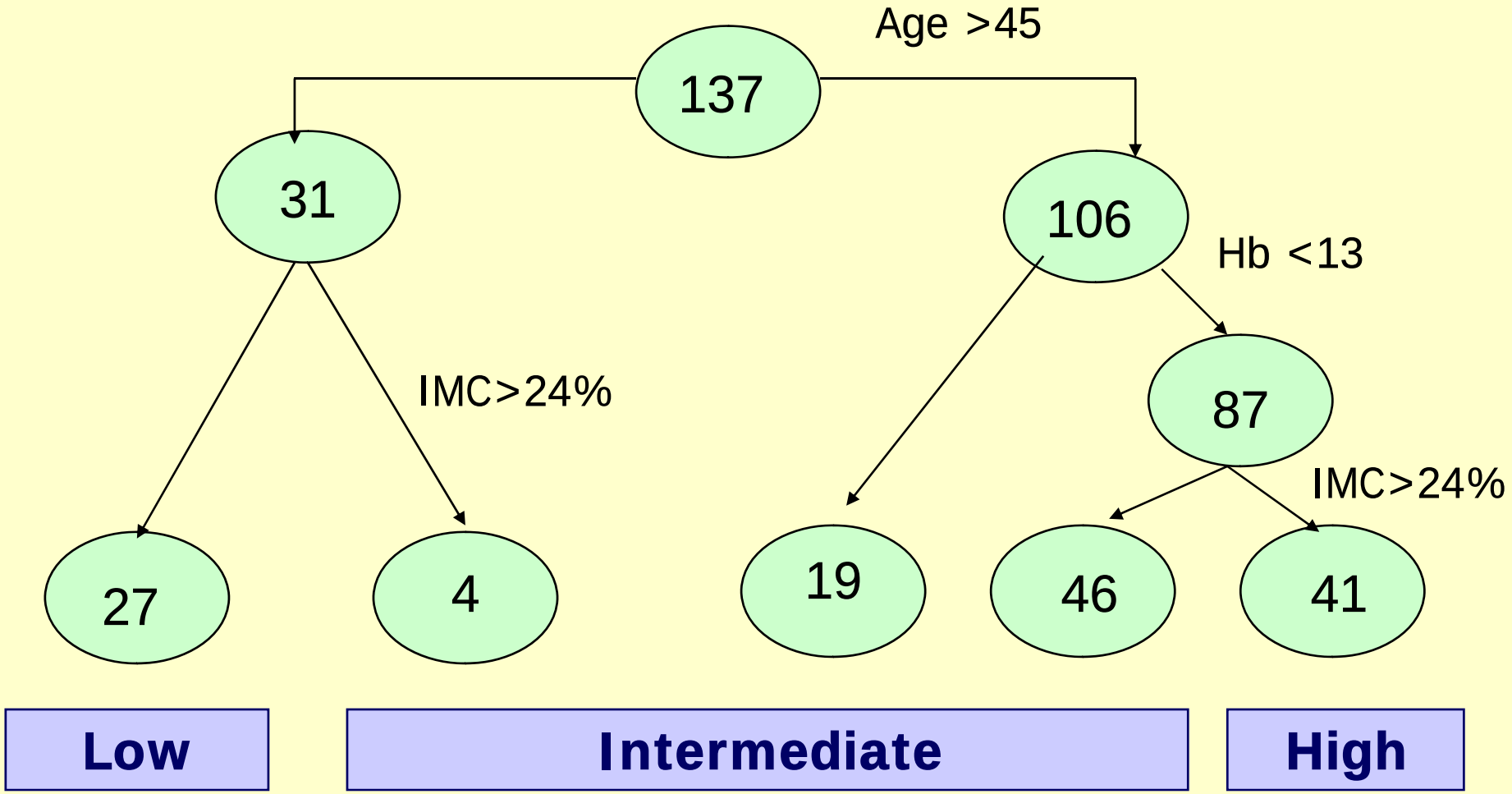
Class II (46%)

- Slightly increased erythropoiesis
- Axial erythropoiesis
- Red cell mass normal or decreased
- Peripheral hemolysis

Class III (12%)

- Erythroid failure
- Decreased red cell volume

Prognostic Classification of Myelofibrosis *(G. Barosi et al, BJH 1988; 137 cases from our Institution)*



Anatomo-clinical Classification of Myelofibrosis

Hyperplastic type (20%)

- Young age (<50)
- High expansion of erythropoiesis
- Mild or no anemia
- Possibly post-polycythemia
- Good prognosis

Dysplastic type (50%)

- Megacaryocytic dysplasia
- Mild anemia
- Intermediate prognosis

Aplastic type (30%)

- Erythroid failure
- Severe anemia
- Bad prognosis

Myelofibrosis: a difficult disease for research

- The disease is rare
- The cases are dispersed
- The disease is undefined

The Italian Consensus Conference for the Diagnostic Criteria of MMM (*Barosi et al. BJH, 1998)*

NECESSARY CRITERIA

A. Diffuse bone marrow fibrosis

B. Absence of Philadelphia chromosome or BCR-ABL rearrangement in peripheral blood cells

OPTIONAL CRITERIA

1. Splenomegaly of any grade
2. Anisopoikilocytosis with tear-drop erythrocytes
3. Presence of circulating immature myeloid cells
4. Presence of circulating erythroblasts
5. Presence of clusters of megakaryoblasts and anomalous megakaryocytes in bone marrow sections
6. Myeloid metaplasia

DIAGNOSIS OF MMM IS ACCEPTABLE IF THE FOLLOWING COMBINATIONS ARE PRESENT

The two necessary criteria plus any other two optional criteria when splenomegaly is present, or plus any other four when splenomegaly is absent

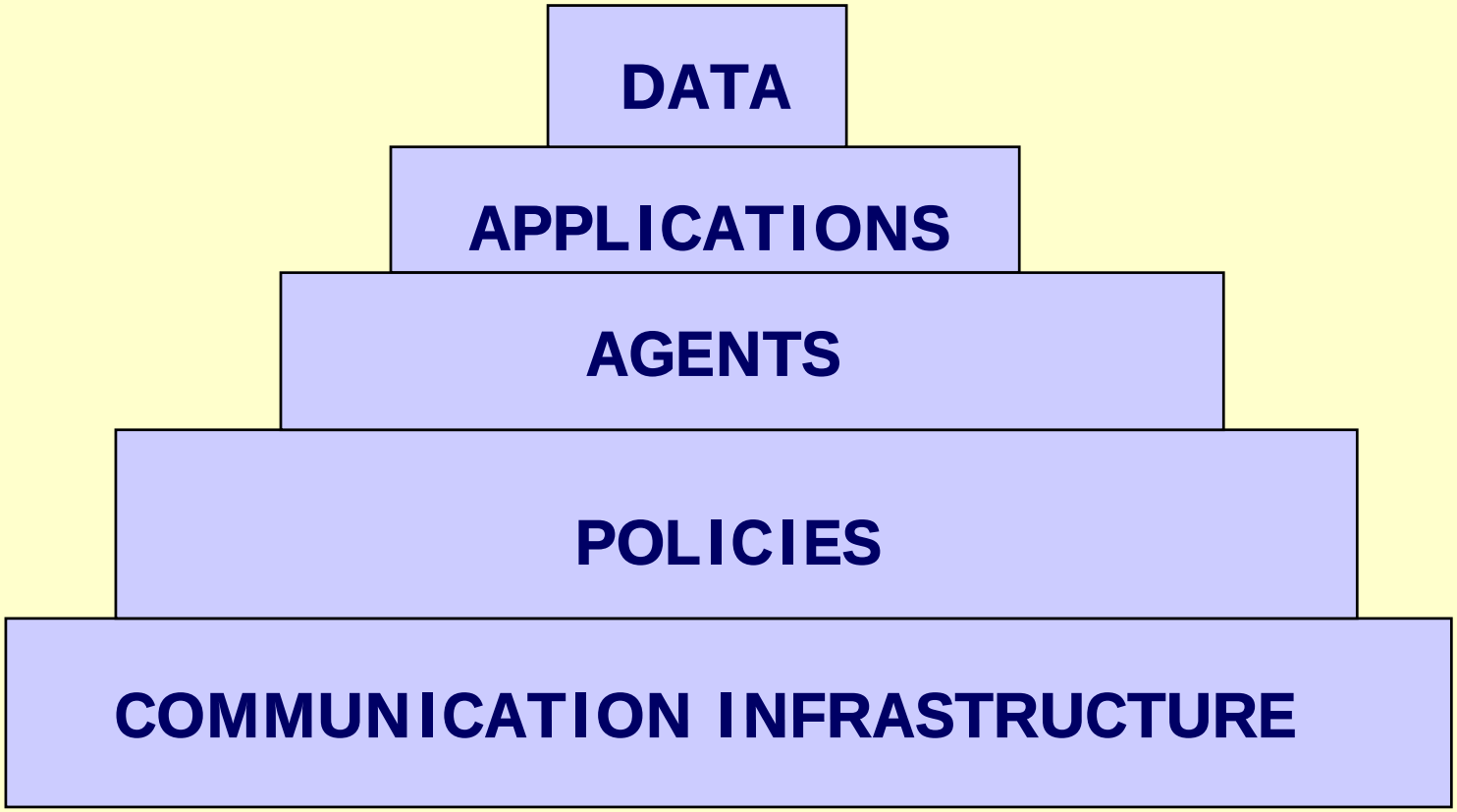
RIMM- An Italian Research Registry for Myelofibrosis with Myeloid Metaplasia (1999-.....)

A permanent organizational structure that maintains a data file for patients who have a specific disease collected within a geographical region with different research objectives

RIMM-Objectives

- Nationwide epidemiology of the disease according with standardized diagnostic criteria
- Process of care of patients with MMM in Italy
- Population-based natural history of the disease
- Population-based outcome of the disease
- New treatment approaches (population-adjusted clinical trials)
- Biology of the disease

RIMM – The Model



RIMM – The Model

1st tier: communication infrastructure (Internet-based applications, prepaid transport and mailing services);

2nd tier: collaborative policies (ownership, liability, intellectual property, confidentiality, security);

3rd tier: agents (coordinating team and participants);

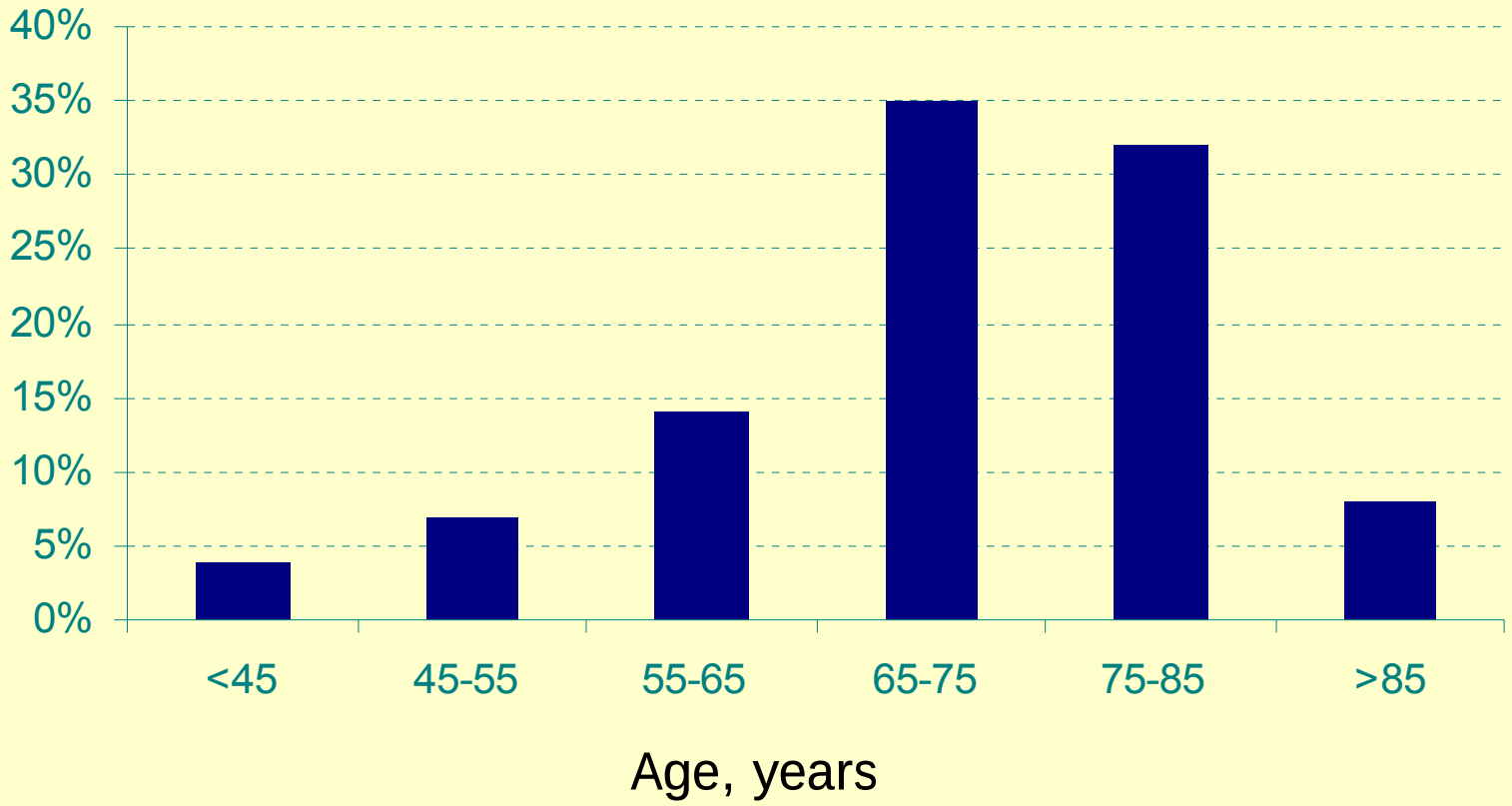
4th tier: applications (research programs and clinical trials implemented in the registry);

5th tier: data (a web-accessible data base for data of all consecutive cases of MMM).

RIMM- Expected-Observed New Cases

- Based on the incidence rates reported in the literature, we expected from 175 to 800 new cases per year
- 1005 patients were registered from June 1999 to September 2004
  - 954 with fully verified diagnostic criteria
  - 51 without the criteria

MMM- Age at diagnosis

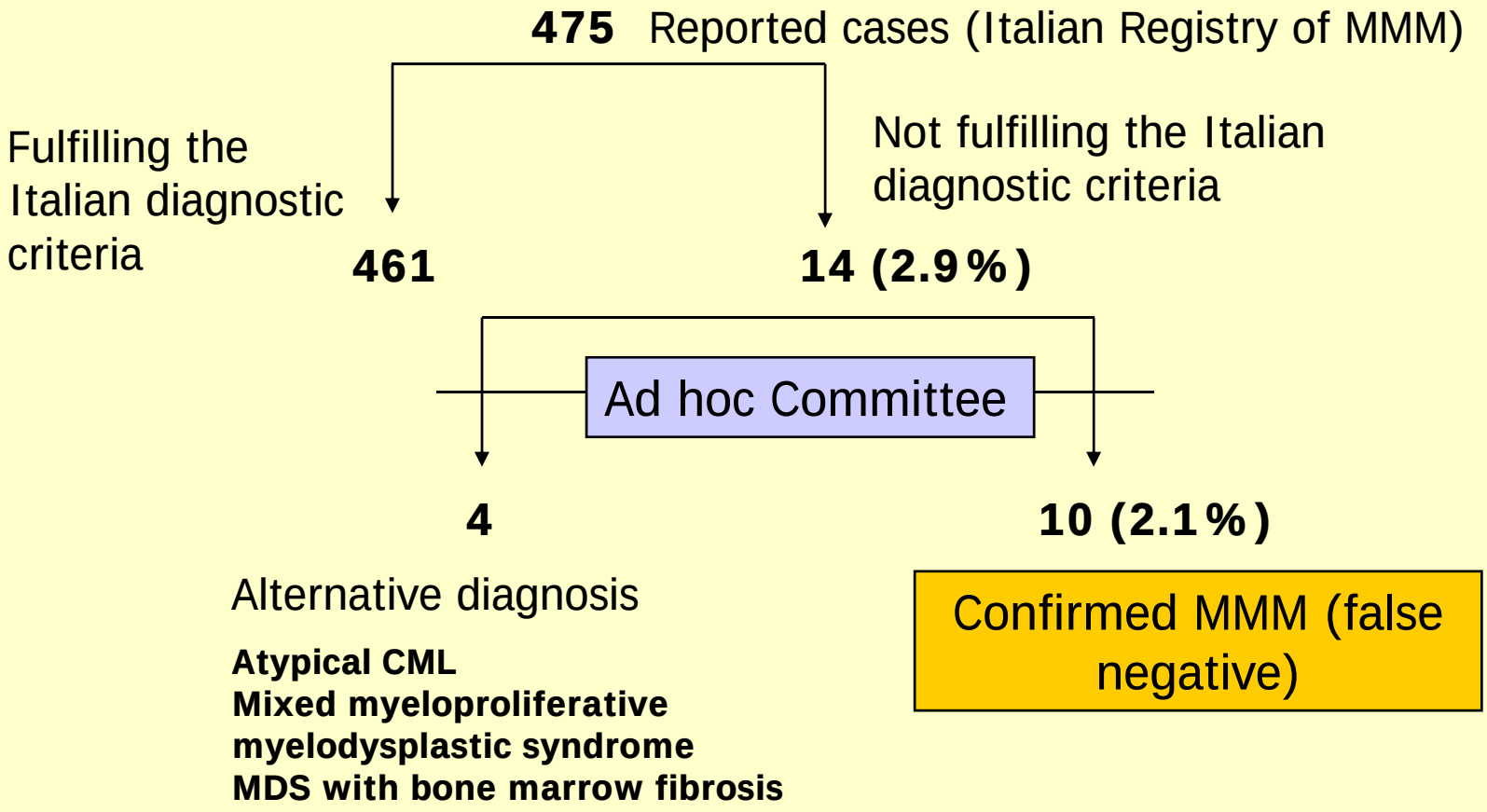


Range = 25-96 yr
Median = 72 yr
Mean = 70 yr

RIMM- Characteristics of the Patients

	From hematologists	From internists
Median age (yrs)	69	76
Previous PV/TE	15%	18%
Spleen > 10 cm from the CM	19%	25%
Leukocytosis (>30)	6%	11%
Severe anemia (Hb < 10g/dL)	14%	21%

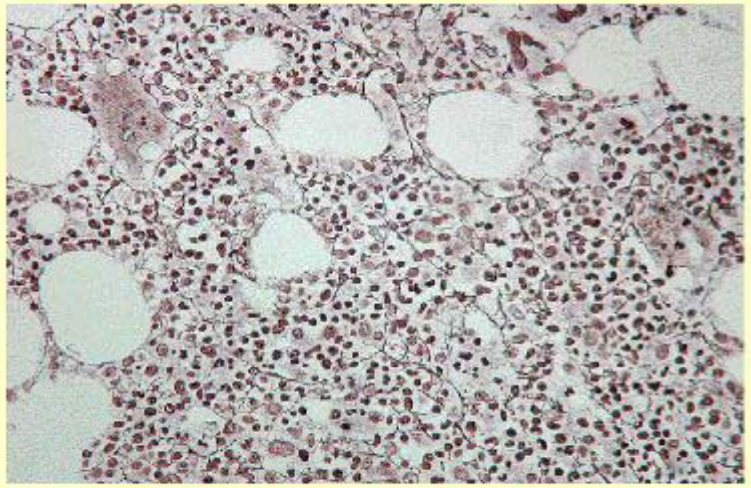
Performance of Italian Diagnostic Criteria

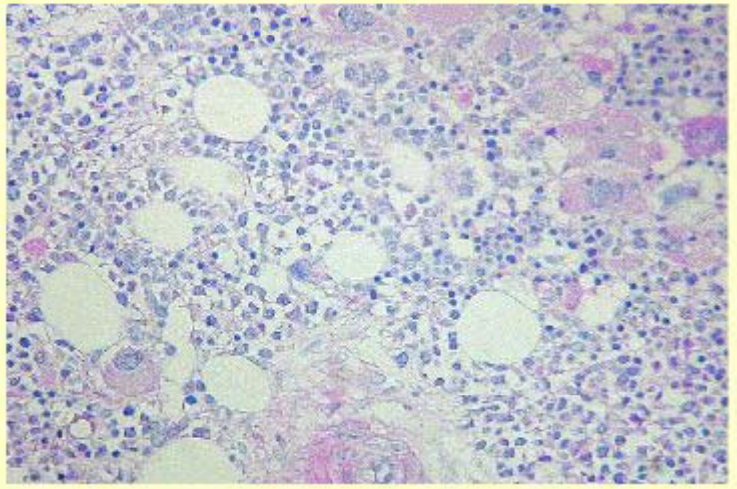


The Diagnostic Challenges of MMM

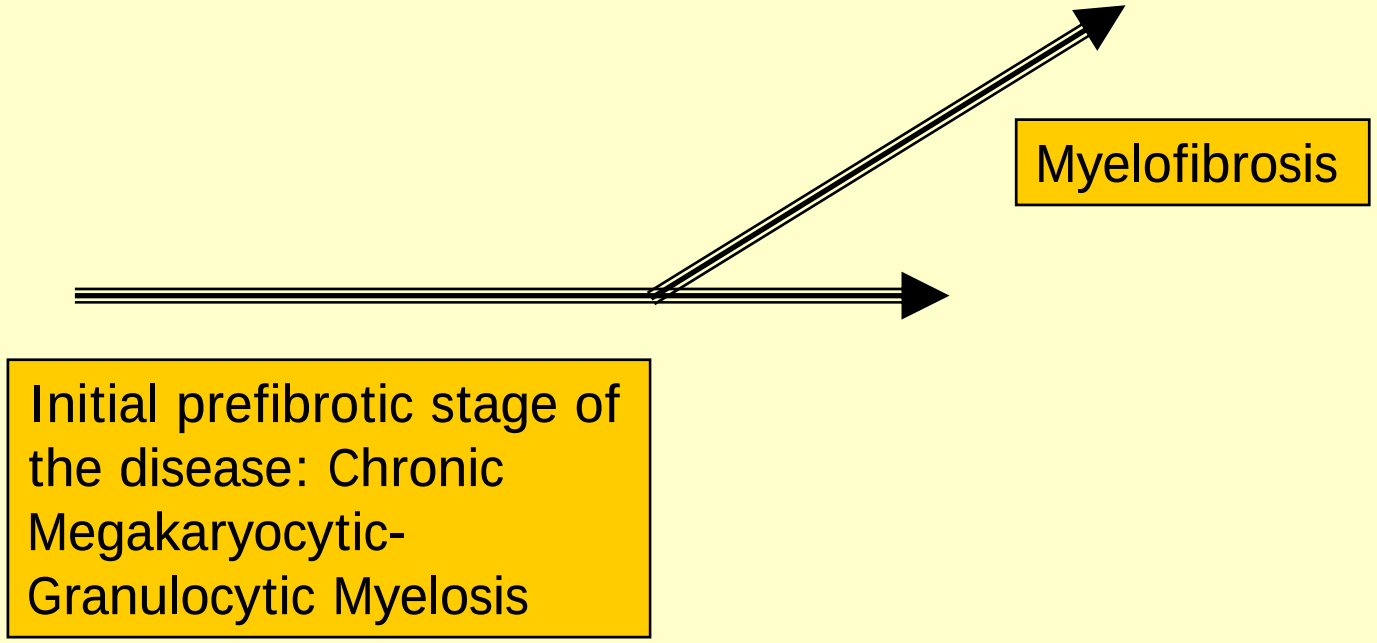
Prefibrotic or early fibrotic myelofibrosis

- Female
- Age: 34 year old
- Splenomegaly: 2 cm from the costal margin
- Hb = 13.4 g/dL
- WBC = 7.5 $x10^9$/L
- Ptl count = 465 $x10^9$/L
- Tear drop cells: +/-
- Immature myeloid cells in PB: 2%
- Bone marrow fibrosis absent
- LDH = 679 (336-1146)
- CD34=11.4 $x10^6$/L



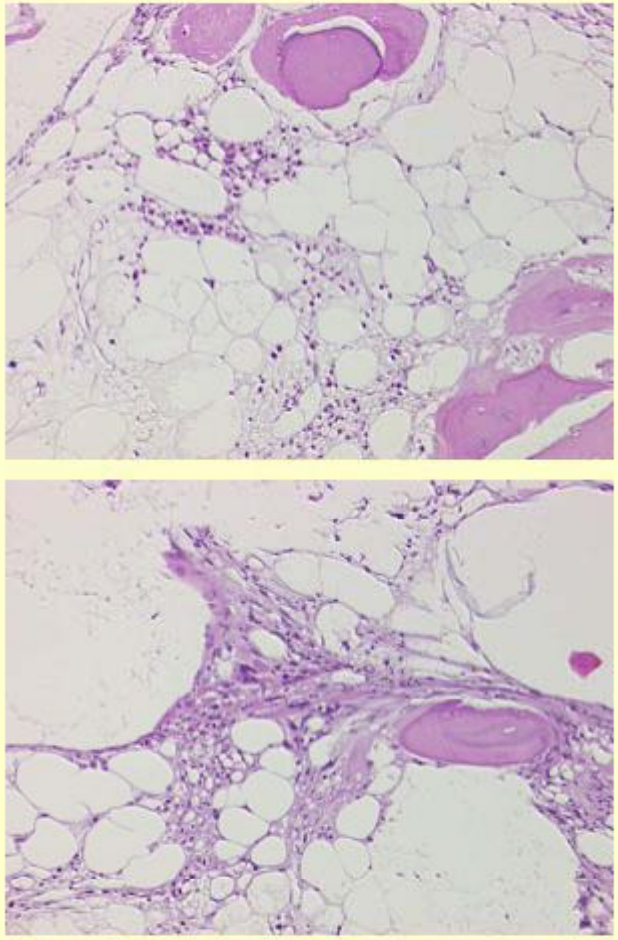


Georgii / Thiele Vision of Myelofibrosis



The Diagnostic Challenges of MMM

Histological variants of MMM: Myelofibrosis with fatty bone marrow



- Severe myeloid hypoplasia
- Dislocation of hematopoiesis in extramedullary sites
- High number of circulating CD34+ cells

- Yamagishi M.,et al. Nippon Ketsueki Gakkai Zasshi. 1984;47:982
- Polino G., et al. Haematologica, 1986;71:117
- Gerli GC., et al. Haematologica, 2001; 86:885
- Rudzki Z., et al. Haematologica. 2003; 88:ELT05.

Diagnostic Criteria for MMM - Conclusion

- The nominalistic approach to the diagnosis is not able today to identify all cases of MMM
- Need of revision of diagnostic criteria
- Need of a better disease stratification

Staging MMM

- To stratify the disease according to **survival**

- To stratify the disease according to **intermediate outcomes** (provide a correspondence between treatment requirements and available therapeutic resources)

- To identify **unusual disease presentations** that may prove to be clinically relevant

Learning sample of patients with Idiopathic Myelofibrosis

- 100 consecutive patients with IMF (post-ET and post-PV excluded) followed at IRCCS Policlinico S. Matteo in Pavia and diagnosed from 1980 to 2000
- M/F =63/37
- Median age = 61.5 yrs (range 16-81 yrs)
- Median follow-up = 48 months (range 16-268 months)
- Splenectomized = 123 (12%)
- Dead = 24 (24%)

Outcome Prediction of Hb >12 g/dL (at Diagnosis)-RIMM cases

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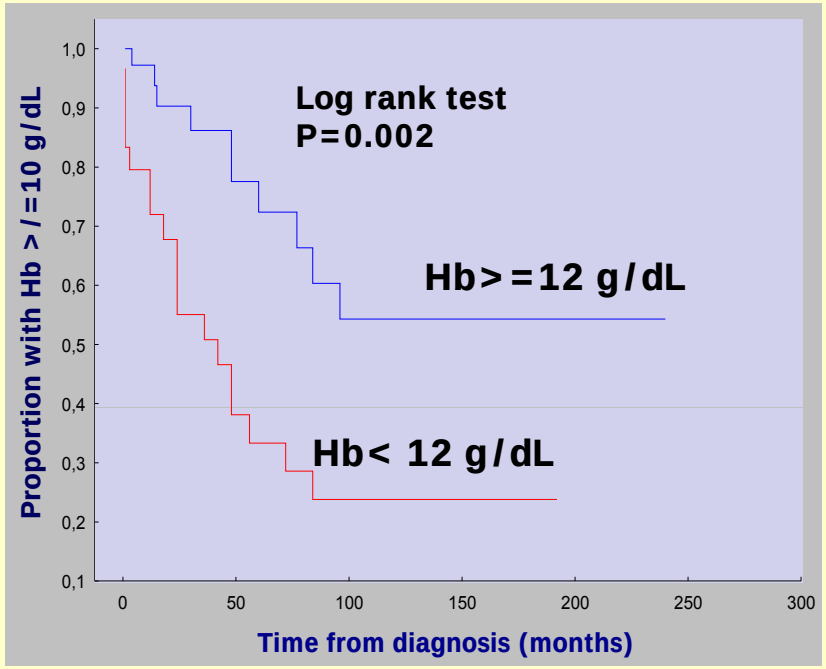
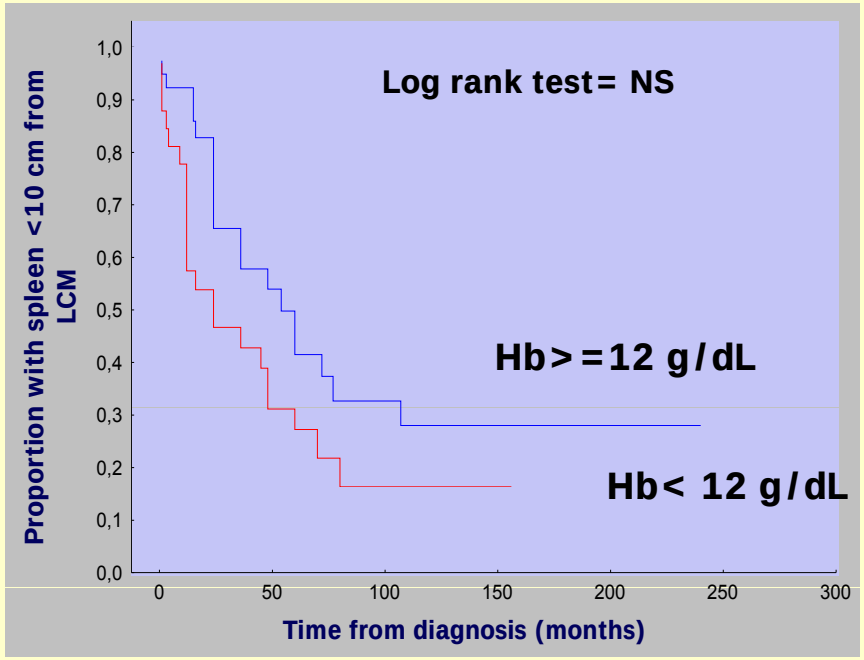


SPLENOMEGALY



Outcome Prediction of WBC >10x10^9/L (at Diagnosis)

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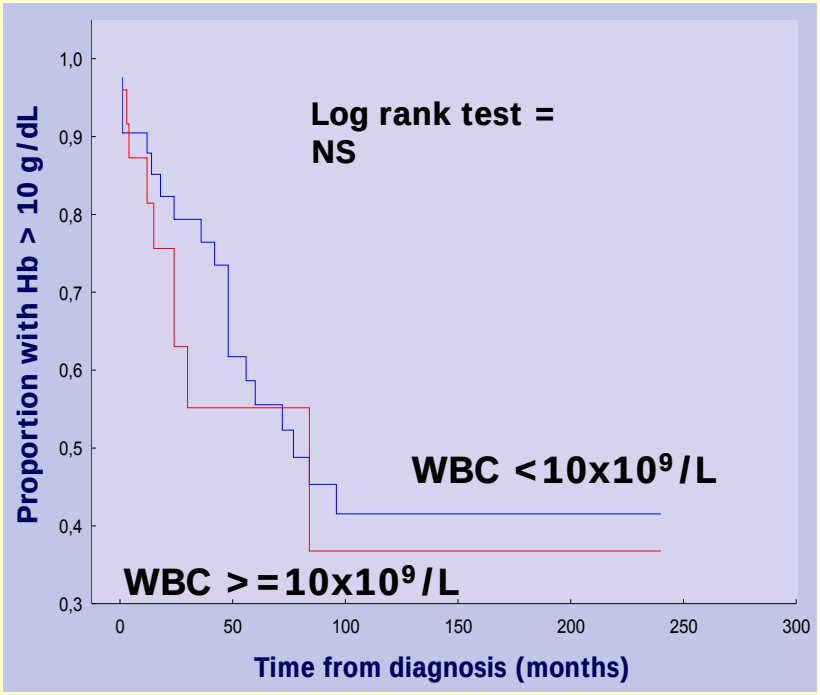

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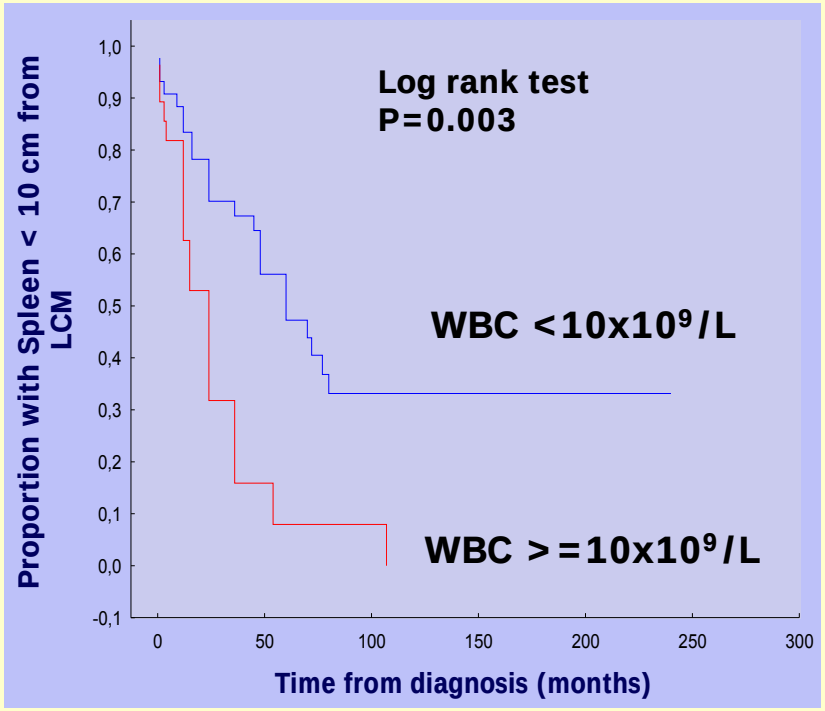

Outcome Prediction of Spleen Volume (at Diagnosis)

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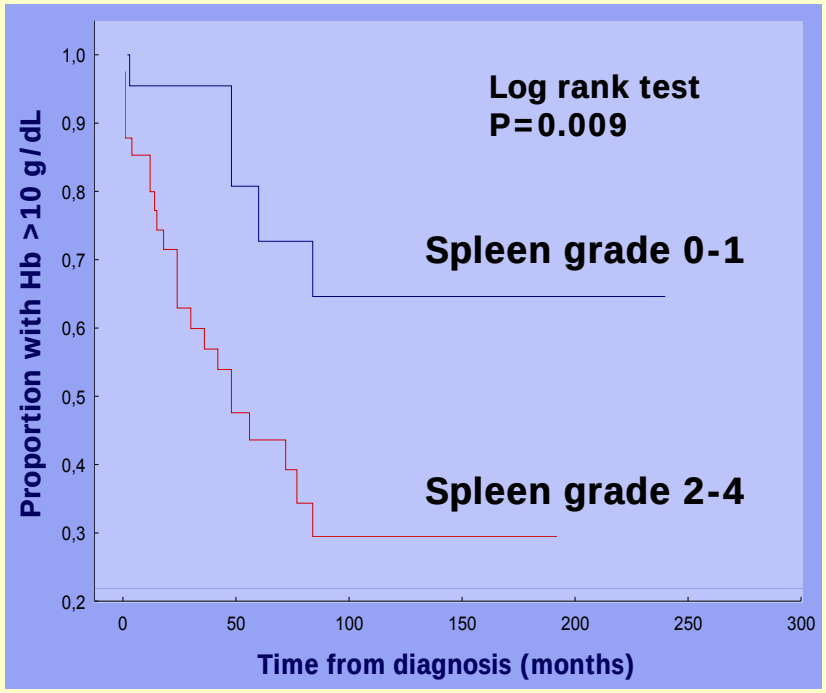

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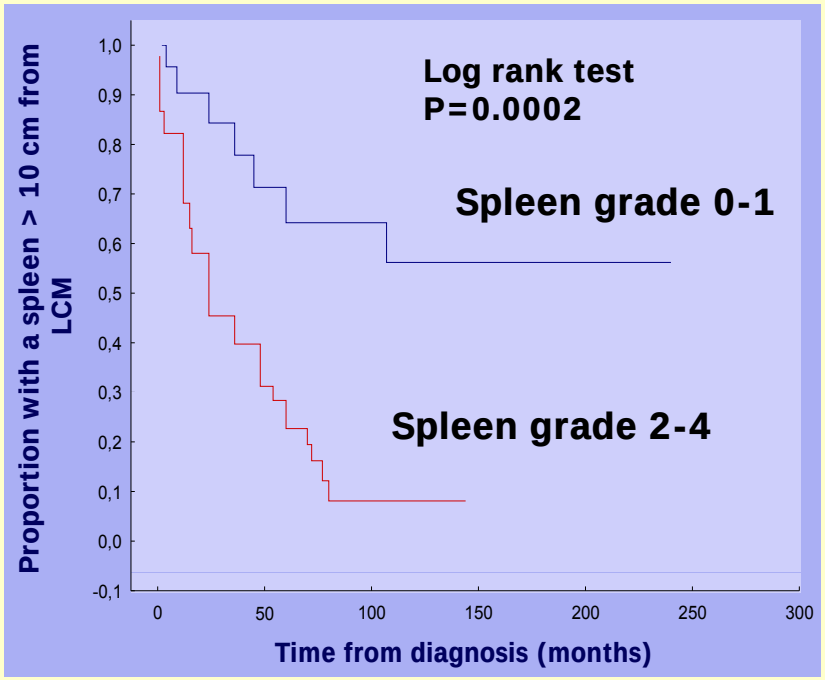

Outcome Prediction of Bone Marrow Fibrosis (at Diagnosis)

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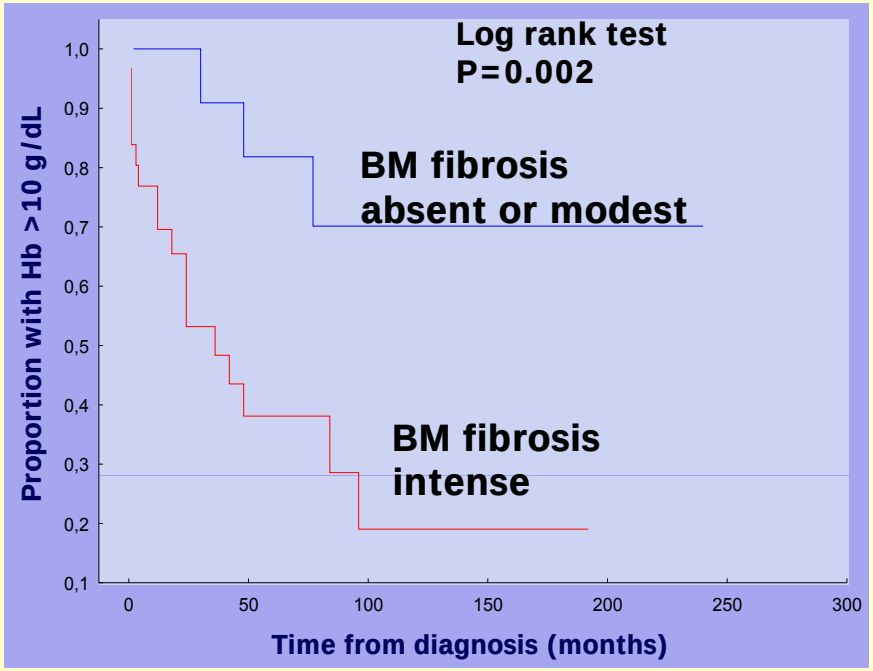

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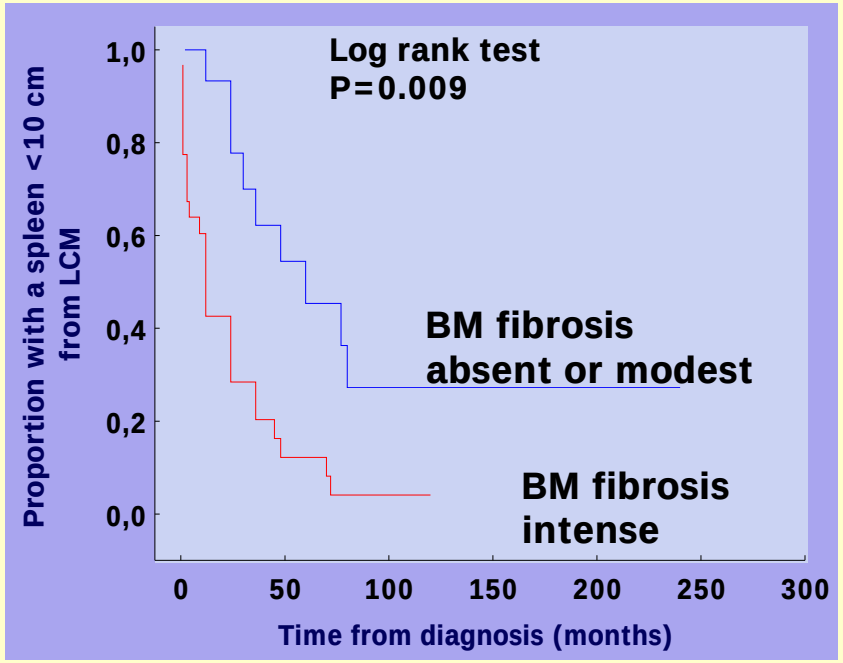

Outcome Prediction of CD34+ Cells >20 x10^6/L (at Diagnosis)

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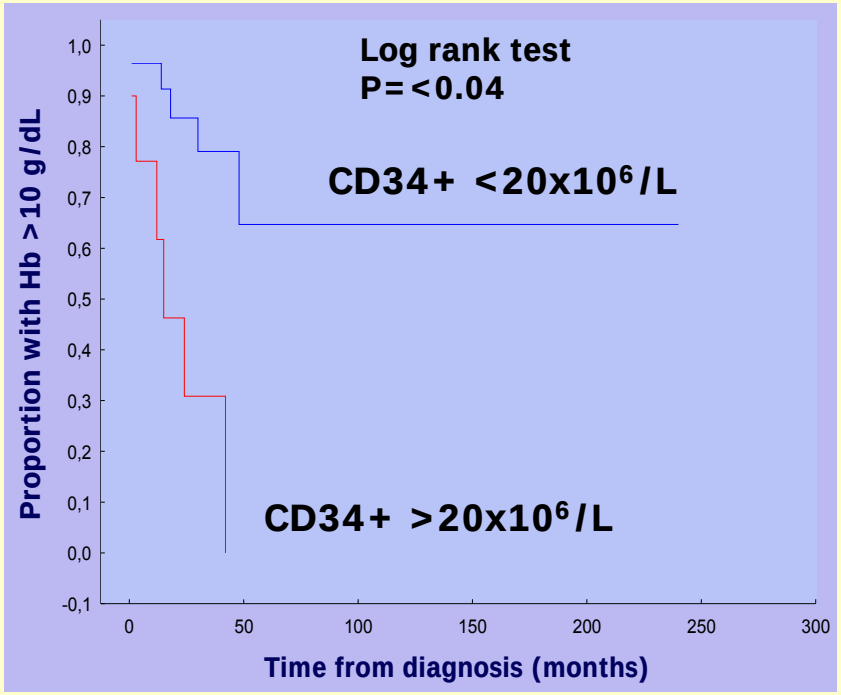

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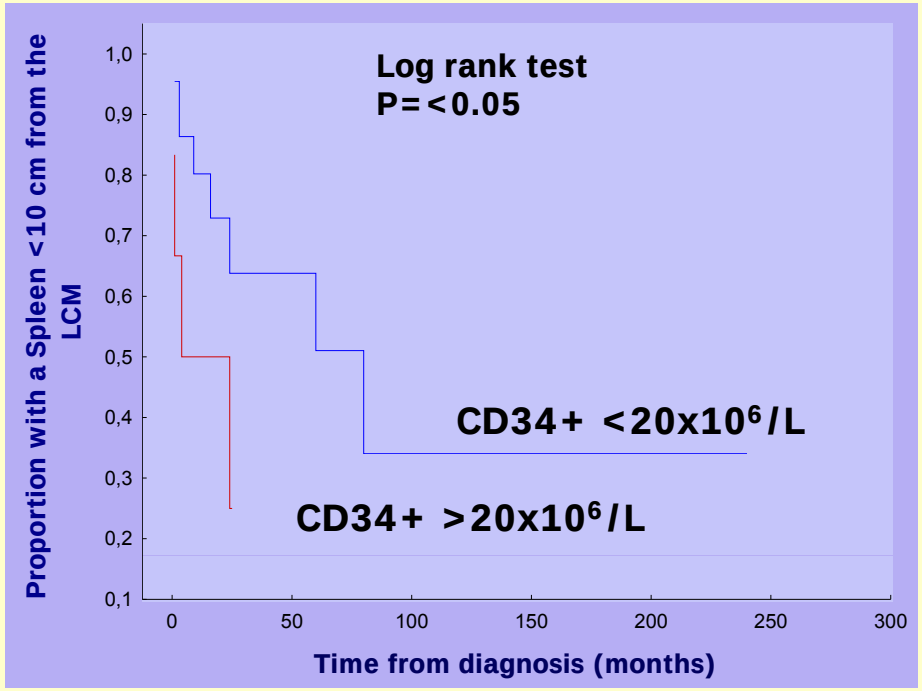
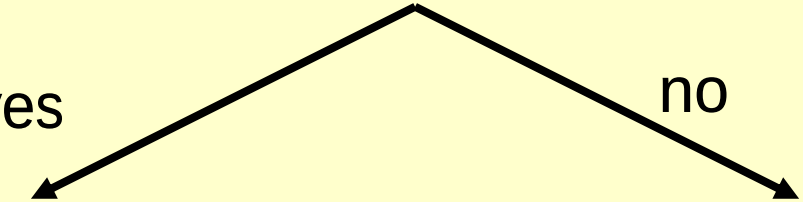


OUTCOME PREDICTION IN MMM

- Hb, spleen volume, bone marrow fibrosis and CD34+ cells at diagnosis predict the development of **anemia**
- WBC, spleen volume, CD34+ cells and bone marrow fibrosis at diagnosis predict the development of **splenomegaly**
- They may be used for staging the disease

Multivariate Prediction (by Regression Cox Model)

- **Hb >12 g/dL, and**
- **WBC <10 x10^9/l and >5 x10^9/L, and**
- **CD34+ cells in peripheral blood <20 x10^9/L**



Neither development of anemia nor splenomegaly (Early stage IMF)

Development of anemia or splenomegaly (Advanced stages of IMF)

Predictive Power of Early Stage IMF

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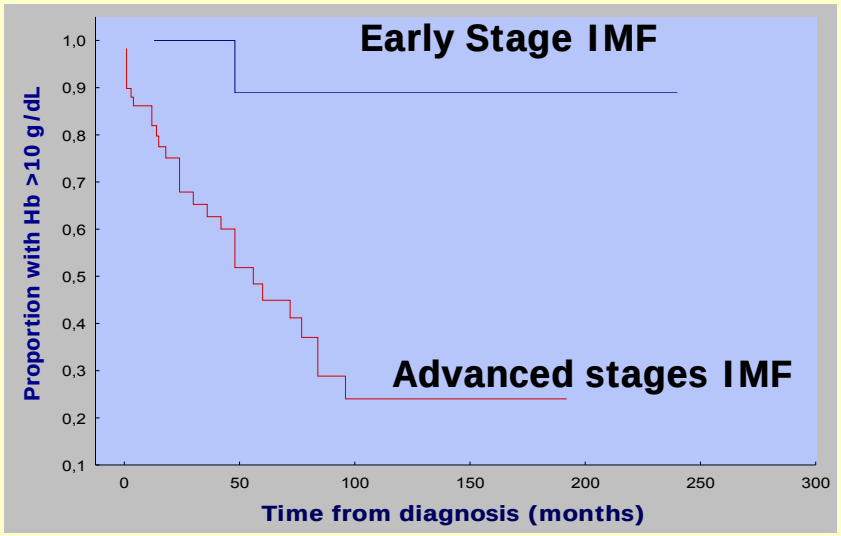
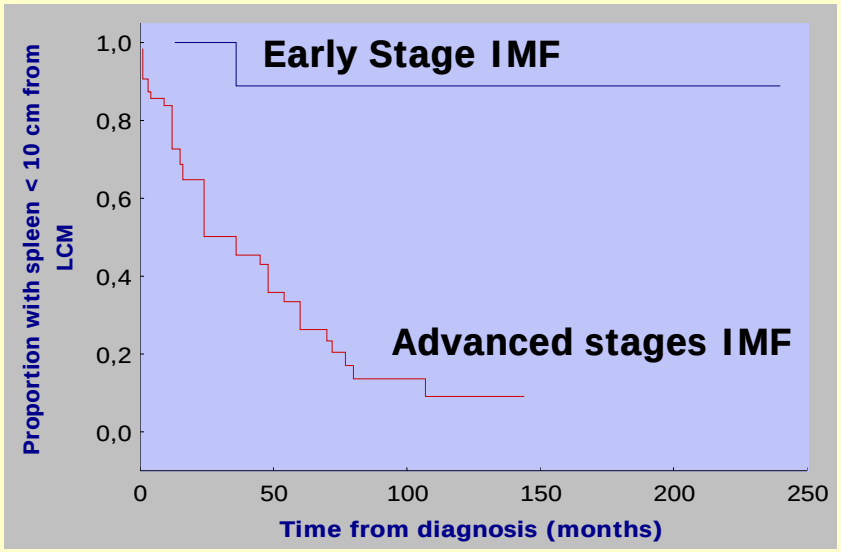
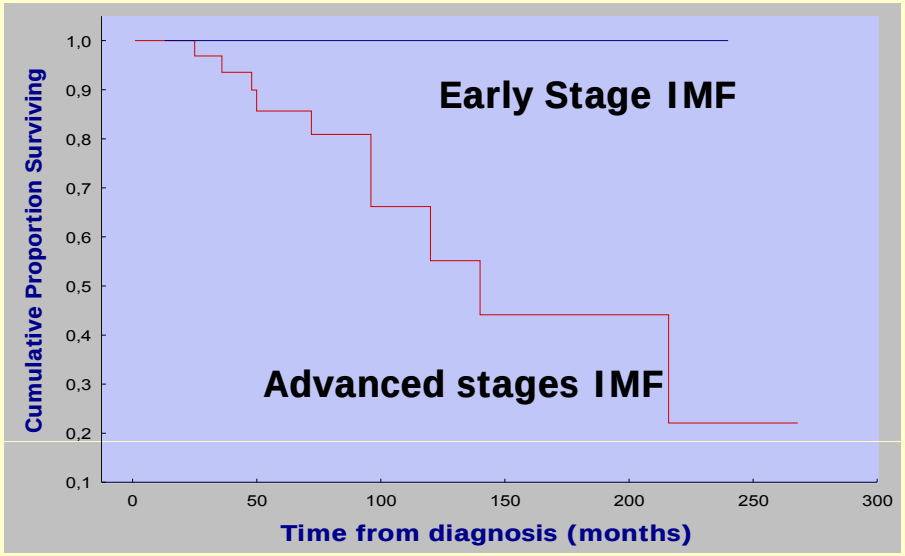


SPLENOMEGALY



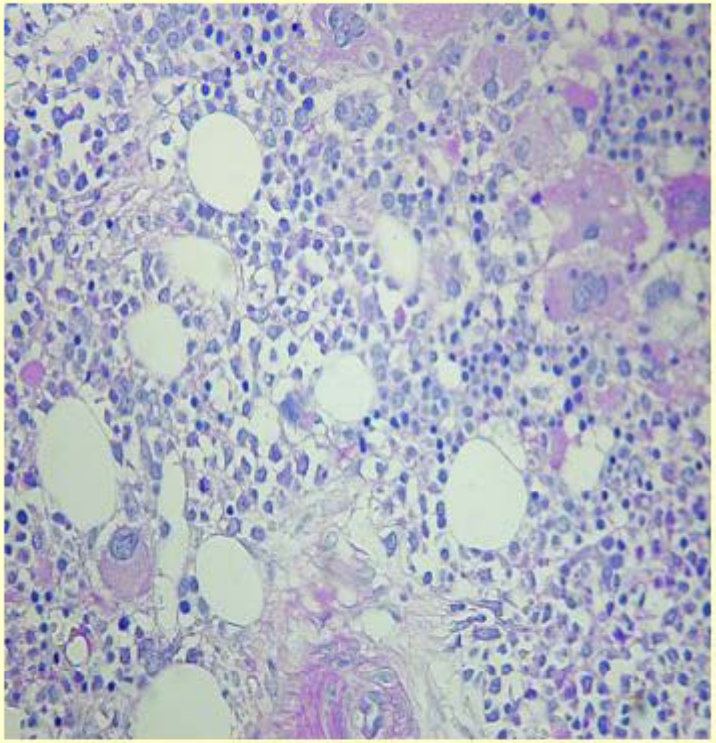
SURVIVAL

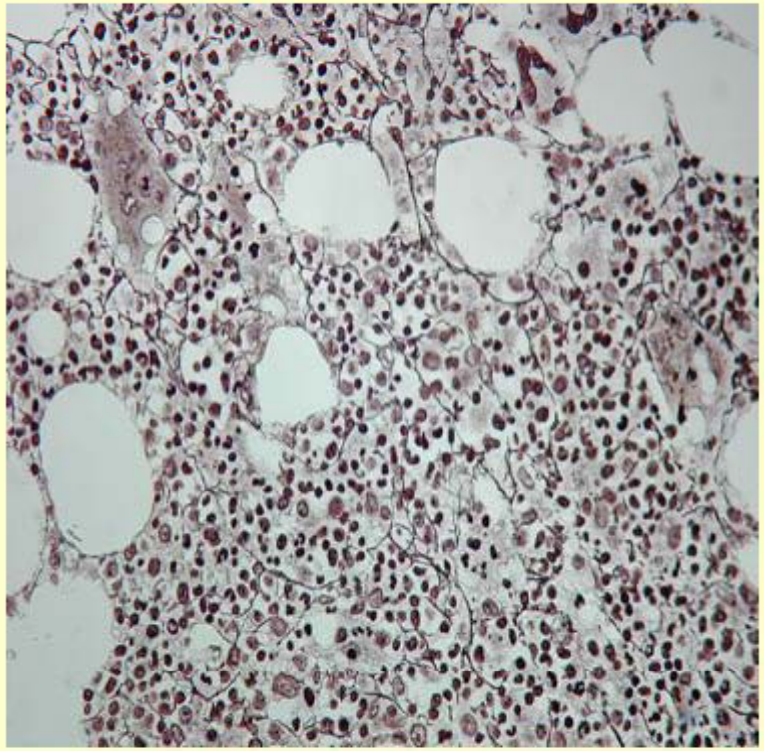


Early Stage IMF (Indolent Myelofibrosis)	Advanced stages IMF
• N= 13 (13%)	• N= 87 (87%)
• Age = 34.5 years (16-72)	• Age = 65 years (19-81)
• M/F = 8/5	• M/F = 58/29
• Splenomegaly absent = 3/13 (23%)	• Splenomegaly absent = 10/87 (11.4%)
• Hb = 13.4 (12-16,4)	• Hb = 12 (6.2-18)
• WBC = 7.5 (5.2-10)	• WBC = 9.1 (2.1-35)
• Ptl count = 465 $x10^9$/L (255-1,100)	• Ptl count = 327 $x10^9$/L (43-1,196)
• Bone marrow fibrosis absent = 2/13 (15.3%)	• Bone marrow fibrosis absent = 3/87 (3.4%)
• LDH = 679 (336-1146)	• LDH = 784 (191-2455)
• CD34=11.4 (range 2.7-20)	• CD34=26.4 (range 1.2-375)
• Splanchnic vein thrombosis at diagnosis =4/13 (31%)	• Splanchnic vein thrombosis at diagnosis = 2/87 (2.3%)

Early Stage IMF (Indolent Myelofibrosis): Possibly Corresponding Syndromes

Prefibrotic Stage of Idiopathic Myelofibrosis (Thiele et al. Leukemia 1999)





Early Stage IMF (Indolent Myelofibrosis): Possibly Corresponding Syndromes

An atypical myeloproliferative disorder with high thrombotic risk and slow disease progression (Barosi et al, Cancer, 1991)

- Young age
- No myeloproliferative evolution
- High tendency to develop thrombosis in atypical sites

Early Stage IMF (Indolent Myelofibrosis): Possibly Corresponding Syndromes

Formes frustes in myeloproliferative disorders (Reid et al, Lancet, 1982)

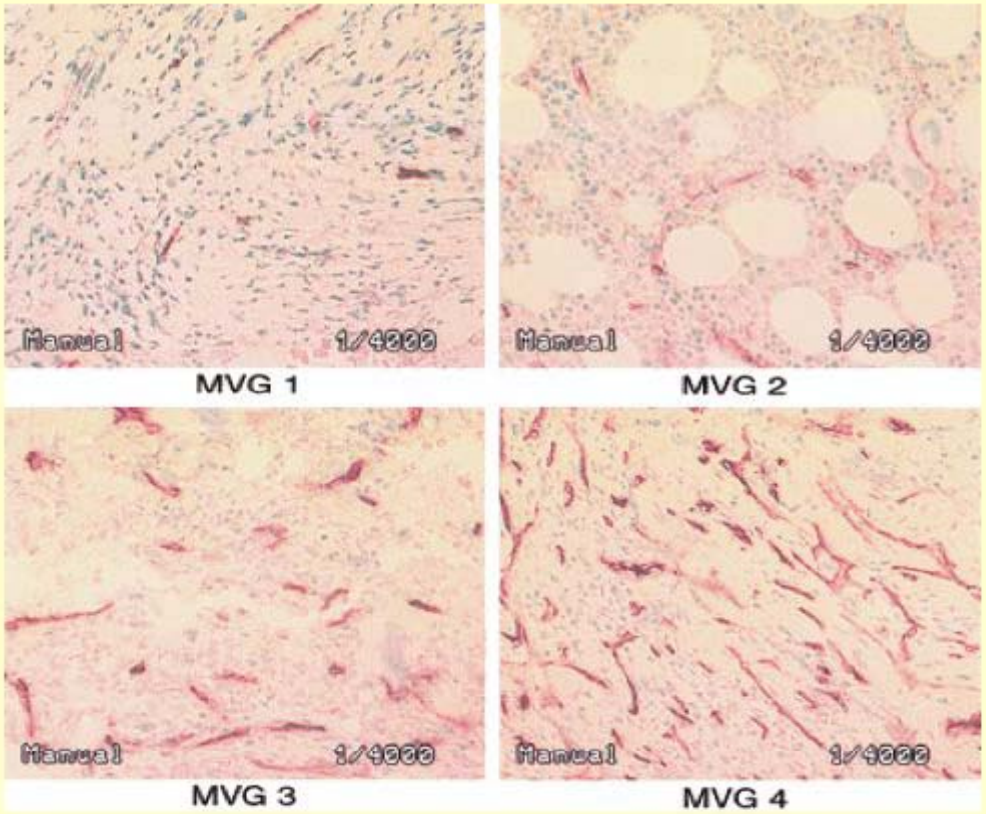
- Peripheral vascular disease
- Slightly elevated platelet count
- Endogenous erythroid colonies

Early Stage of Idiopathic Myelofibrosis

- An early stage of IMF exists
- This has been probably overlooked (misdiagnosis, no symptoms)
- It does not completely correspond to the “prefibrotic” picture of the disease
- In some case it takes the form of a very indolent disease (more than 10 years without evolution)
- Diagnostic criteria?
- Biology?

Angiogenesis in MMM

- Angiogenesis in bone marrow of patients with MMM results from normal to highly increased



CD34+ immunostaining

Mesa et al, Blood 2000;96:3374

Angiogenesis in MMM

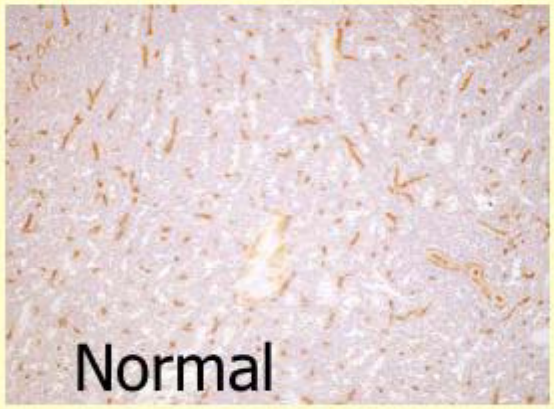
- Marrow vascularity in patients with MMM is substantially increased compared with normals and patients with PV and ET

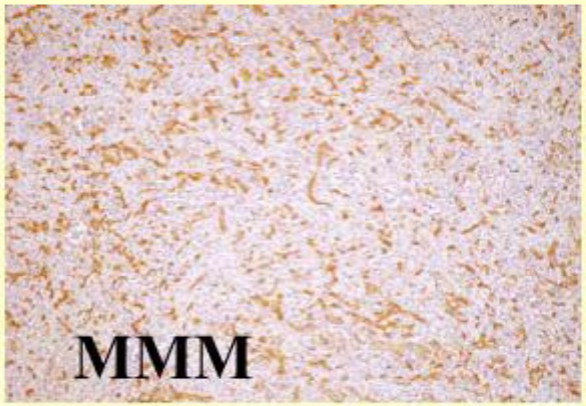
Visual MVD grade	MMM (N=114)	Normals (N= 44)	PV (N=15)	ET (N=17)
1 (Normal)	1.8%	77.3%	26.7%	23.5%
2	28%	22.7%	40%	64.7%
3	37.7%	0%	33%	12%
4	32.5%	0%	0%	0%

Mesa et al, Blood 2000;96:3374

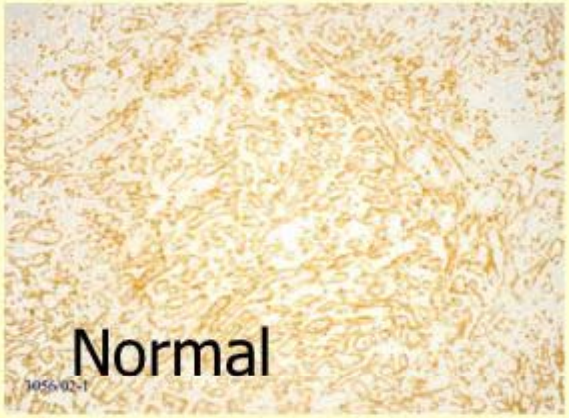
Angiogenesis in MMM

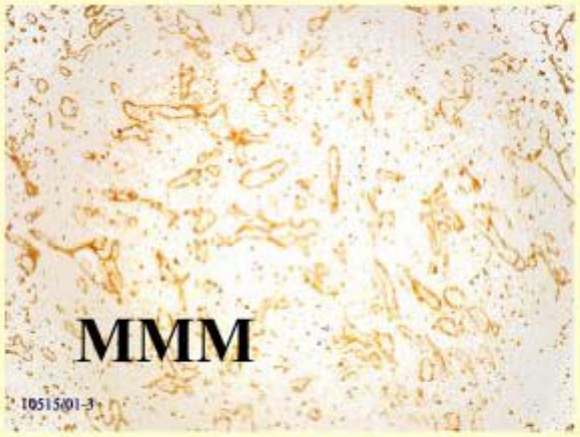
- Increased angiogenesis (CD34+ microvessel staining) is also a feature of the spleen in patients with MMM





CD34+ microvessel staining



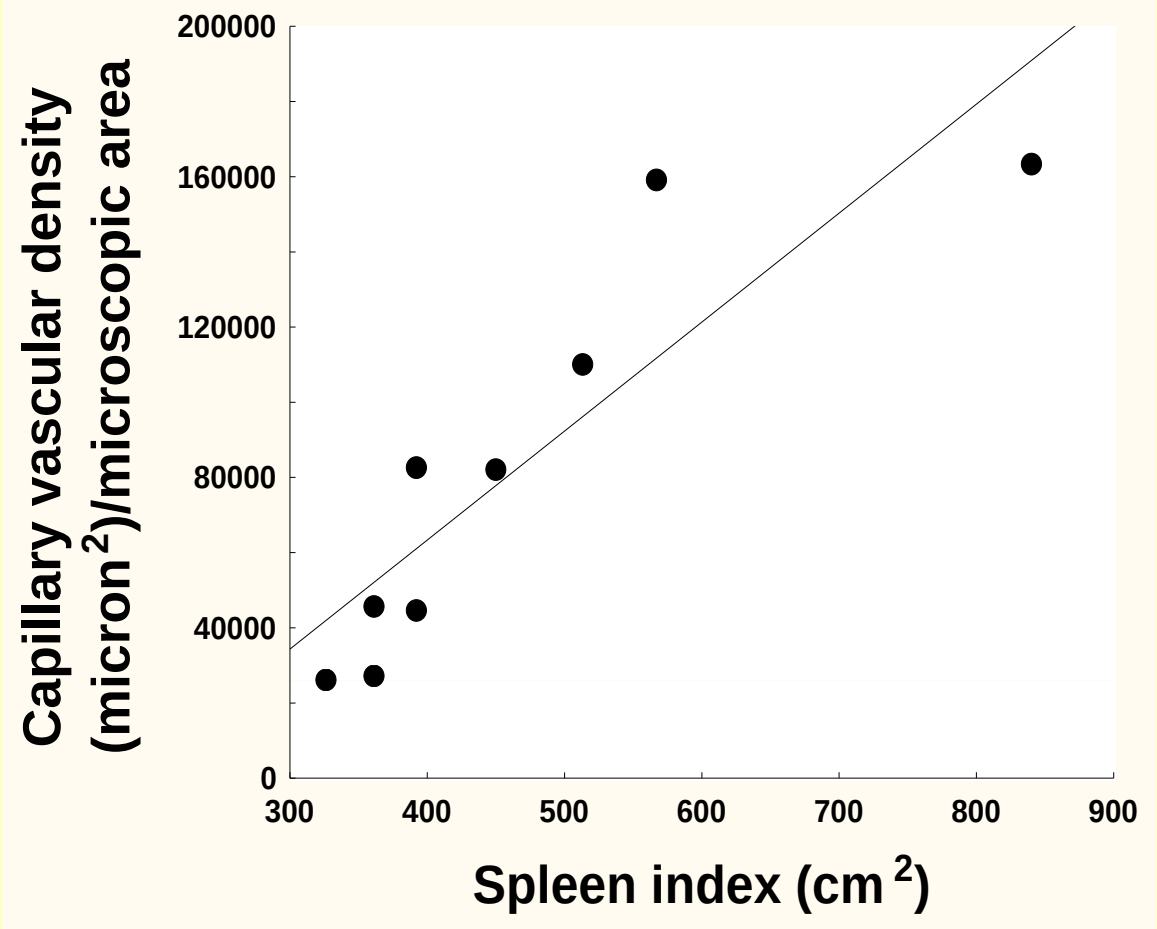


CD8+ sinusoid staining

Barosi et al. BJH 2004

Angiogenesis in MMM

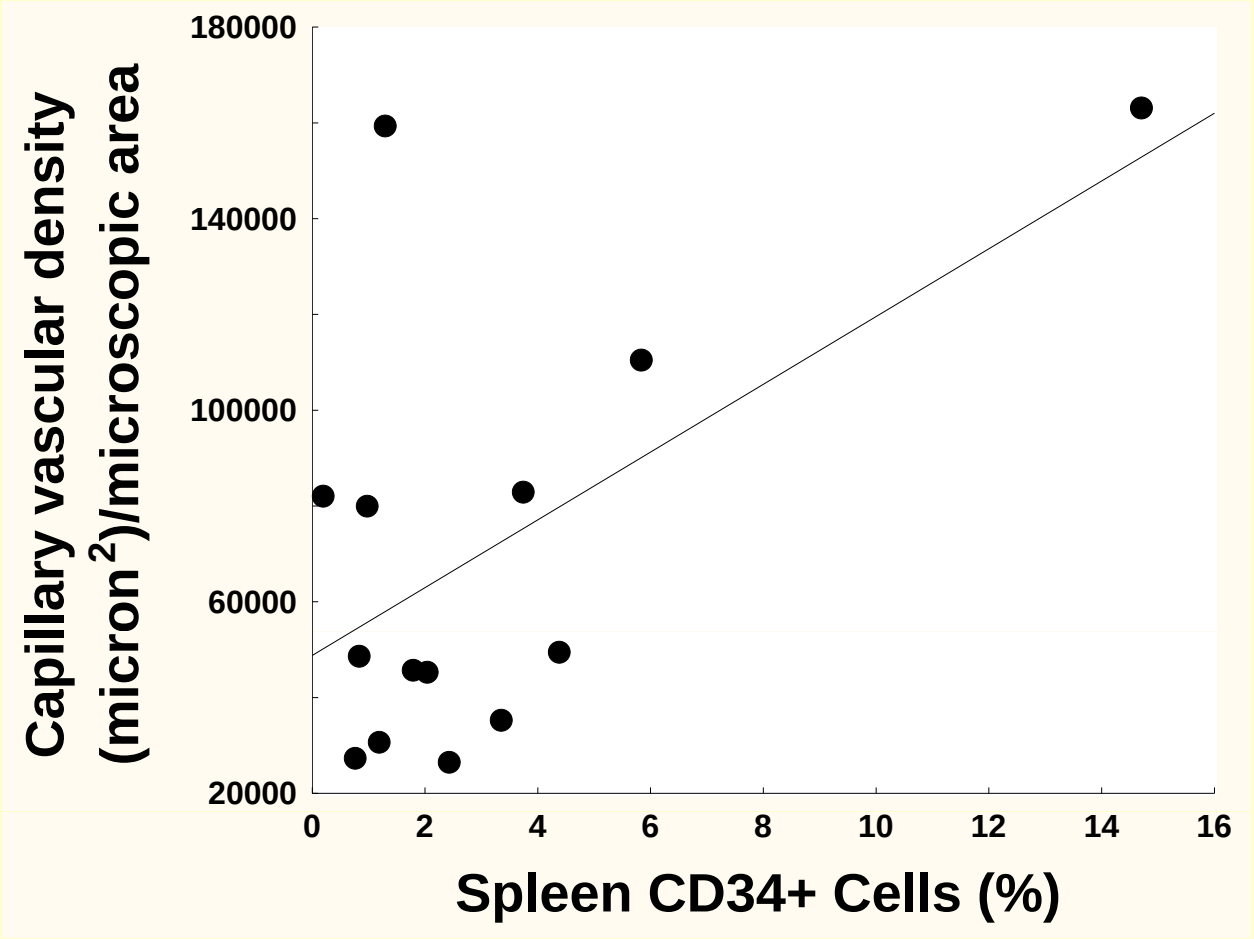
- Capillary microvessel density in the spleen of patients with MMM correlates with spleen size



Barosi et al. BJH 2004

Angiogenesis in MMM

- Capillary microvessel density of the spleen of patients with MMM correlates with the extent of myeloid metaplasia



Barosi et al. BJH 2004

Pro-angiogenic Factors in Myelofibrosis

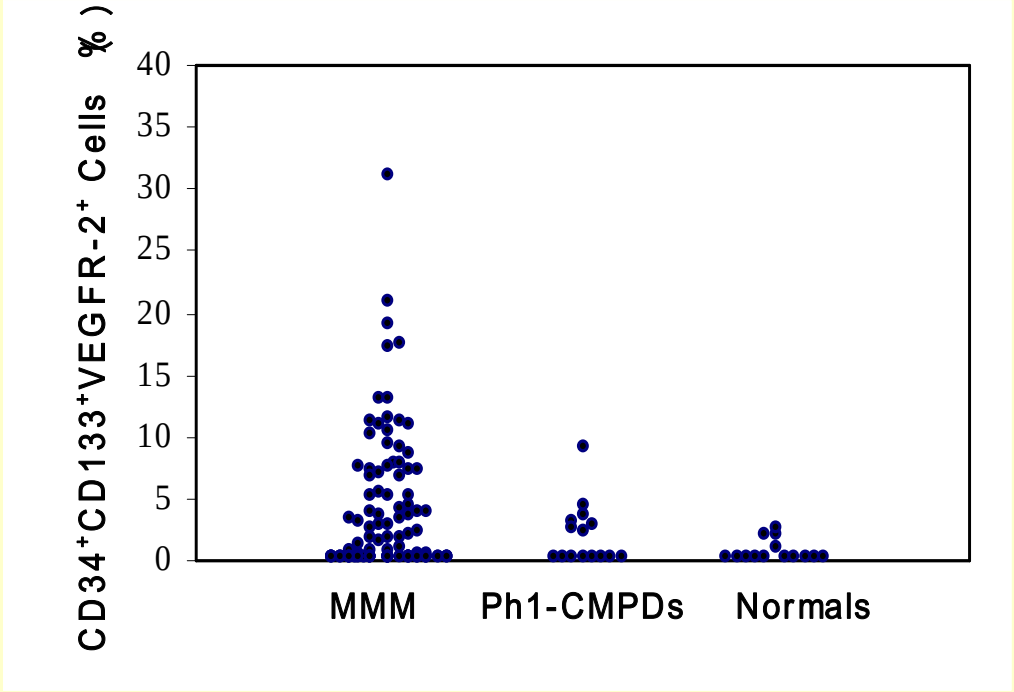
- Elevated expression of plasma and BM VEFG, b-FGF and TGF-β is consistently documented in MMM
- Poor evidence of their pathogenetic role in angiogenesis of MMM

Angiogenesis – cellular mechanism

- During development, new vessels are formed by differentiation of progenitor cells (endothelial progenitor cells or angioblasts)
- In adult life, endothelial progenitor cells circulate in normal subjects and are increased in patients with vascular injury (trauma, myocardial infarction, angina..)
- In some tumors, mature endothelial cells may bear the same cytogenetic aberration of hematopoietic progenitor cells (CML, lymphoma)

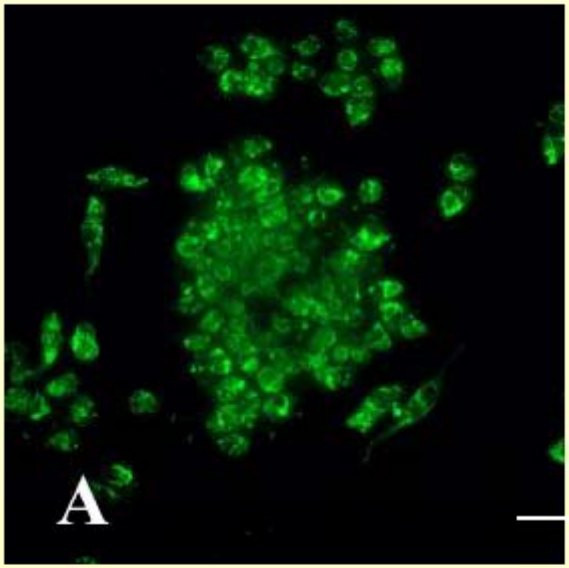
Endothelial Progenitor Cells (EPC) in Myelofibrosis

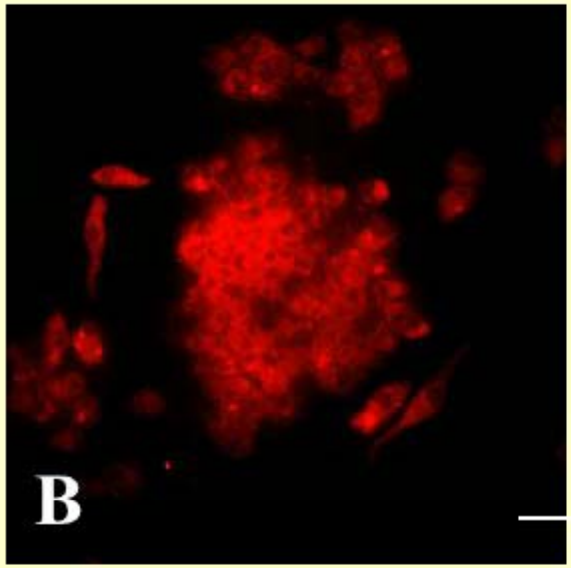
- The phenotypically characterized circulating EPC are consistently elevated in a significant proportion of MMM patients



Rosti ASH 2003

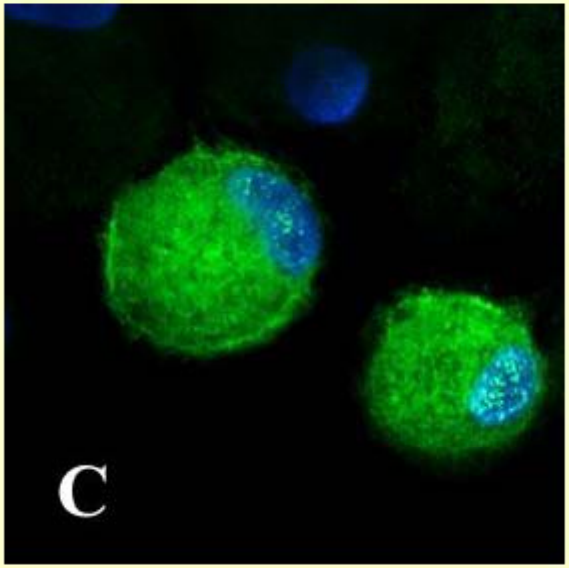
Endothelial colonies

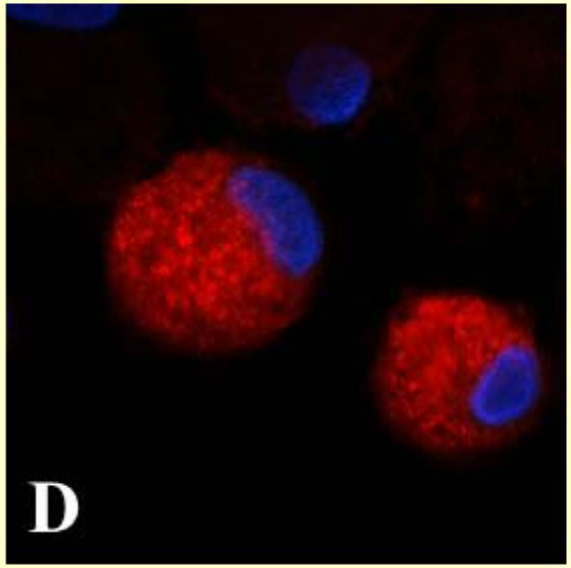




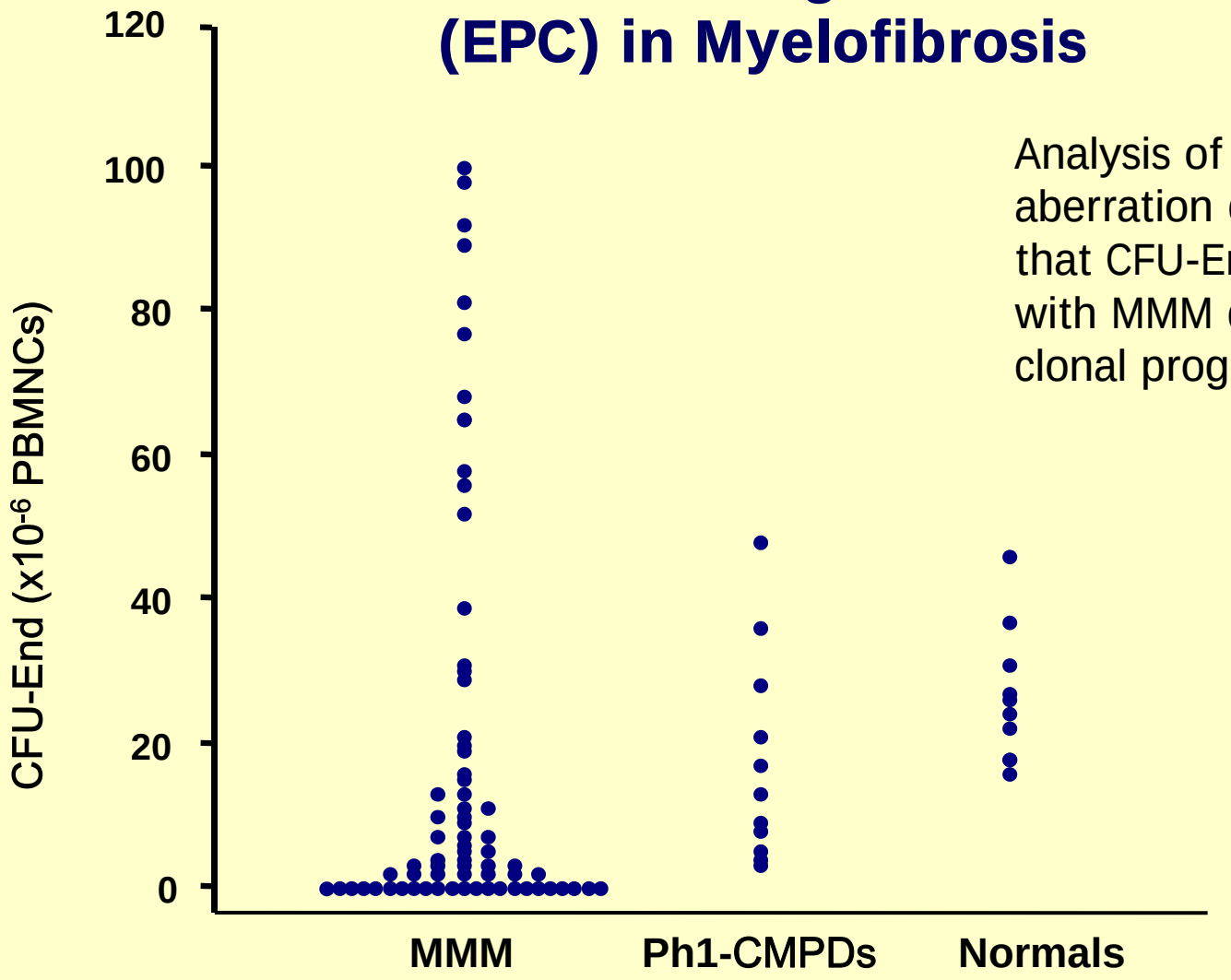
Ve-cadherin = green

CD31 = red





Endothelial Progenitor Cells (EPC) in Myelofibrosis



Analysis of chromosomal aberration documents that CFU-End of patients with MMM derive from a clonal progenitor cell

Endothelial Progenitor Cells (EPC) in Myelofibrosis - Conclusions

- Increased number of EPCs (immunophenotype and endothelial cell colture) is a distinctive characteristic of MMM
- Circulating EPCs belong to the malignant clone
- New mechanisms for angiogenesis may be hypothesized